Mini Review

HOX Genes as Potential Markers of Circulating Tumour Cells

Richard Morgan and Mohamed El-Tanani

Institute of Cancer Therapeutics, Faculty of Life Sciences, University of Bradford, UK

Running title: *HOX* Genes and CTCs

Keywords: Circulating tumour cells; *HOX* gene; biomarker

Abbreviations

CTC, circulating tumour cell

EpCam, epithelial cell adhesion molecule

Abstract

Circulating tumour cells (CTCs) have significant diagnostic potential as they can reflect both the presence and recurrence of a wide range of cancers. However, this potential continues to be limited by the lack of robust and accessible isolation technologies. An alternative to isolation might be their direct detection amongst other peripheral blood cells, although this would require markers that allow them to be distinguished from an exceptionally high background signal. This review assesses the potential role of *HOX* genes, a family of homeodomain containing transcription factors with key roles in both embryonic development and oncogenesis, as unique and possibly disease specific markers of CTCs.

Introduction

Circulating tumour cells (CTCs) have been identified for a wide range of cancers types, although notable examples include breast [1], prostate [2], colorectal [3], and lung cancer [4]. In addition to providing a mechanistic explanation for widespread disease dissemination, they also represent a potential biomarker for diagnosis and prognosis. Hence, for example in breast cancer the presence of CTCs is an independent prognostic factor for both progression-free survival and overall survival, and a cut-off of 5 CTCs / 7.5 ml has been used to identify patients with a good or poor prognosis [5]. In addition, the persistence of CTCs after adjuvant therapy for breast cancer is significantly associated with a shorter disease-free survival.

Although CTCs are a potentially useful biomarker, an intrinsic difficulty of this approach is their rarity, and their identification requires a combination of enrichment and detection strategies. The latter is based on technologies that can distinguish CTCs from blood cells based on their physical (e.g. size, density, electrical charge and deformability), and biological properties (e.g. cell surface protein expression and viability). Ficol gradients are one of the simplest and earliest methods used to separate CTCs, but other available methods include the use of dielectrophoretic technology to exploit differences in the biophysical properties of CTCs and blood cells [6], and label-free chips that separate CTCs based on their size [7]. Most techniques used to enrich CTCs are based on the expression of distinct cell surface markers that can be immunologically detected. The most commonly exploited cell surface marker is the epithelial cell adhesion molecule (EpCam), which is central to a range of antigen-capture based techniques such as CellSearch® and CTC-CHIP [8, 9]. However, there are no uniformly expressed CTC markers, and EpCam itself is not present on all CTCs, most notable because it is down regulated during the epithelial-to-mesenchymal transition, a process that is central to metastatic change [10].

An alternative to CTC isolation could be the identification of gene expression patterns that are not present in blood cells. This would inevitably be complicated by the vast excess of RNA from white blood cells in any preparation made from whole blood. However, sensitive detection methods for identifying a small number of transcripts, for example quantitative PCR (qPCR), could allow CTCs to be identified, as well as potentially the cancer from which they originated, if specific patterns of gene expression could be established that are not presented by white blood cells. One particularly strong candidate in this respect is the *HOX* gene family.

***HOX* genes**

The *HOX* genes are a family of homedomain-containing transcription factors that have a crucial role in the patterning of the early embryo, as well as some more limited functions in adult physiology. There are 39 *HOX* genes in the human genome present in 4 separate genomic clusters, named A-D, which arose from a series of duplication events prior to the emergence of mammals [11]. Unusually, genes within each cluster share enhancer regions, leading to a closely coordinated pattern of expression during development and a close relationship between the position of each *HOX* gene in the cluster and its temporal and spatial expression. Thus, for example, the 3' most member of the HOXB cluster, *HOXB1*, is expressed earlier and more anteriorly than one of the most 5' members, *HOXB9* [12]. HOX proteins have relatively limited specificity for DNA, binding to a 4 base pair sequence. Their binding specificity and hence target gene activation (or in some cases repression) is greatly enhanced by their interaction with specific co-factors such as PBX and MEIS proteins [13, 14].

During early development, *HOX* gene products help to define the identity of the tissues in which they are expressed, principally along the anterior to posterior axis. These tissues include the nervous system posterior to the midbrain, the gut, and the limbs [11]. In addition, some adult cells continue

to express *HOX* genes and in these cells *HOX* expression is necessary to maintain cellular identity, and in some cases is also needed for continued cell proliferation and survival. One of the best characterized examples is the hematopoietic stem cell and its differentiated progeny. *HOXB4* is required for the continued proliferation of hematopoietic stem cells, and once their daughter cells subsequently begin to differentiate through the long cascade of hematopoietic progenitor cell types they express different *HOX* genes in nested patterns, with the mature lymphocytes expressing quite distinct patterns of *HOX* genes compared to the parental stem cells [15].

HOX genes in cancer

In addition to their function in the embryo and in some normal adult cells, the *HOX* genes also have a key function in malignancy. This has been reviewed extensively elsewhere, but in brief, the *HOX* genes are frequently dysregulated in almost all of the solid and haematological malignancies in which they have been studied [16]. A number of them have been shown to function as oncogenes, including *HOXA9*, the forced expression of which in normal mouse bone marrow cells leads to acute myeloid leukaemia within 3 months [17]. Many other *HOX* genes have been shown to have a pro-oncogenic role in terms of supporting tumour growth. HOX proteins can promote cell proliferation and survival through interaction with the PBX co-factor, and the disruption of HOX/PBX dimer formation using the HXR9 peptide has been shown to trigger apoptosis in a wide range of solid malignancies both *in vitro* and *in vivo [18-23]*. Furthermore, *HOX* expression has been shown to promote a number of aggressive tumour characteristics, including invasion and migration [24], DNA repair [25], and the induction of angiogenesis [26]. Consequently, the tumour expression of a number of *HOX* genes has been shown to be related to the survival of cancer patients [27].

Despite a number of excellent reviews on this subject, the *HOX* expression profiles of different cancer types generally remains unclear as it is difficult to directly compare the information in studies that have used different techniques to assess *HOX* expression, and some studies have given conflicting results. This review attempts to establish a broad overview of *HOX* expression in different solid malignancies by only including data on the over- or under-expression of *HOX* genes based on at least 2 different techniques (e.g. QPCR and immunohistochemistry), and which has been demonstrated in 2 different studies. The findings of this review for head and neck [24, 28-36], oesophageal [37, 38], gastric [39, 40], colorectal [41], pancreatic [42], hepatic [41], lung [43-46], breast [47], bladder [48, 49], renal [23], prostate [50, 51], and ovarian cancer [52-54] are summarized in Fig 1 and Table 1. It is striking that different tumour types show very different patterns of *HOX* expression. For example, gastric tumours tend to show very little *HOX* gene over-expression, whilst hepatic tumours exhibit a vastly dysregulated network of *HOX* genes. It also seems clear that different tumour types have radically different *HOX* gene expression patterns, with the notable exception of bladder and prostate tumours (Fig 1). These distinct, tumour-specific expression profiles make *HOX* genes potential markers of CTCs.

HOX genes as CTC markers

As discussed above, CTCs generally make up only a very small proportion blood cells. After excluding red blood cells, the majority of remaining cells are lymphocytes and monocytes. Both have previously been shown to express *HOX* genes, and indeed the expression of all 39 *HOX* genes has been studied in different lymphocyte and monocyte populations [55]. This revealed that monocytes, T-lymphocytes and B-lymphocytes expressed distinct but also overlapping sets of *HOX* genes, and also that *HOX* gene expression changed considerably upon activation of the cell types. In contrast, very little is known of *HOX* expression in other myeloid lineages, although previous studies have

suggested that *HOX* genes are generally down-regulated in mature blood cells [56]. In the context of possible CTC markers, these data are potentially very useful as they also reveal that a number of *HOX* genes are not expressed in normal lymphocytes or monocytes (Table 2). Despite the reduced number of *HOX* genes available for possible CTC identification, all tumour types appear to present with a distinct pattern of non-lymphocytic/monocytic *HOX* expression, except for bladder and prostate tumours (Fig 2). Based on these findings, it seems possible that *HOX* expression profiles could act as a "fingerprint" of specific tumour types, potentially allowing CTC detection without CTC isolation. For example, hepatic CTCs might be distinguished by their very high level of HOX dysregulation, whilst gastric CTCs appear to be characterized by high levels of HOXA13 expression in the absence of other HOX genes. Ovarian and pancreatic cancer is likewise distinguishable by the predominant expression of genes from the HOXB cluster, although the former expresses more posterior (5') *HOX* genes than the latter. It should be noted that the data presented in this review are inevitably incomplete with respect to the expression of *HOX* genes in both cancer and normal blood cells. However, it is becoming apparent that there is high degree of differential HOX expression between mature blood cells and CTCs, pointing towards a nucleic acid based methodology for distinguishing between these cell types.

Table 1: *HOX* gene expression in cancer – stages and comparators

***HOX* genes**	**Cancer**	**Disease stage**	**Comparator**	**Reference**
HOXB9	Head and neck		Immortalized normal oral keratinocytes, normal oral mucosa	[24]
HOXA1	Head and neck		Normal oral mucosa	[28]
HOXB7	Head and neck	I, II, III, IV	Normal oral mucosa	[29]
HOXD10	Head and neck	Primary, metastatic	Immortalized normal oral keratinocytes, normal oral mucosa	[30]
HOXB5	Head and neck	N0, N+	Normal oral mucosa	[31]
HOXA10	Head and neck	I, II, III, IV	Normal oral tissue	[32]
HOXD10	Head and neck		Immortalized normal oral keratinocytes, normal oral mucosa	[33]
HOXC5	Head and neck		Rat normal oral tissue	[34]
HOXC6	Head and		Normal tissue	[35]

	neck			
HOXA10, A13, B7, C4, C8, D9, D10, D13	Oesophageal cancer	IIa, IIb, III	Normal oral mucosa	[37]
HOXD9	Oesophageal cancer		None	[38]
HOXA13	Gastric cancer	All T stages, metastasis	Normal mucosa	[39]
HOXC6	Gastric cancer		Normal mucosa	[40]
HOXA9, B3, B8, B9	Colorectal cancer	pT1, pT2, pT3, pT4	Normal colorectal mucosa	[41]
HOXA3, A5, A6, A7, A9, A10, A11, A13, B1, B6, B7, B8, B9, B13, C5, C6, C8, C9, C10, C11, C12, C13, D1, D3, D4, D8, D9, D10	Hepatic cancer	pI, pII, pIII, pIVa	Normal liver tissue adjacent to the tumour	[41]
HOXB2, B5, B6, B7	Pancreatic cancer	Pre-malignant pancreatic intraepithelial neoplasia, adenocarcinoma	Normal tissue	[42]
HOXA1, A5, A10, C6	Non-small cell lung cancer	pTNM: pT2(N0/1/2)M0 pStage: IB/IIB/IIIA	Normal tissue	[43]
HOXB9	Lung cancer	All clinical stages and TNM categories	Normal tissue	[46]

		assessed		
HOXA1	Small cell lung cancer	Extensive and limited disease	Normal lung alveoli epithelium	[45]
HOXA6, A13, B2, B4, B5, B6, B7, B8, B9, C5, C9, C13, D1, D8	Breast cancer		Non-malignant tissue	[47]
HOXB13	Bladder cancer	Muscle invasion	Non-invasive disease	[49]
HOXC4, C5, C6, C11	Bladder cancer		Normal uroepithelium	[48]
HOXB13	Prostate cancer		Normal prostate tissue	[50]
HOXC4, C5, C6, C8	Prostate cancer	Primary tumours, metastatic disease	Normal prostate epithelium, benign hypertrophy	[51]
HOXA10	Ovarian cancer		Normal ovarian tissue	[52]
HOXB7, B13	Ovarian cancer		Normal ovarian tissue	[53]
HOXB8	Ovarian cancer	Histological stages: high, low FIGO stages: IIIc, IV	Normal ovarian tissue	[54]
HOXA5, A9, B5, C9, D8, D9, D10	Renal cancer		Normal adjacent tissue	[23]

Table 2: *HOX* genes not expressed in normal lymphocytes or monocytes

HOXA	**HOXB**	**HOXC**	**HOXD**
HOXA1	*HOXB1*	*HOXC5*	*HOXD4*
HOXA7	*HOXB2*	*HOXC9*	*HOXD13*
HOXA9	*HOXB3*	*HOXC10*	
HOXA11	*HOXB5*	*HOXC11*	
HOXA13	*HOXB6*		
	HOXB7		
	HOXB8		
	HOXB9		
	HOXB13		

Figure 1: Summary of *HOX* gene expression in different solid malignancies.

Figure 2: Summary of *HOX* genes expressed in different solid malignancies that are not expressed in normal lymphocytes or monocytes.

References

1. Toss A, Mu Z, Fernandez S, Cristofanilli M. CTC enumeration and characterization: moving toward personalized medicine. Ann Transl Med 2014; 2: 108.
2. Moreno JG, Miller MC, Gross S*, et al.* Circulating tumor cells predict survival in patients with metastatic prostate cancer. Urology 2005; 65: 713-8.
3. Cohen SJ, Punt CJ, Iannotti N*, et al.* Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol 2008; 26: 3213-21.
4. Tanaka F, Yoneda K, Kondo N*, et al.* Circulating tumor cell as a diagnostic marker in primary lung cancer. Clin Cancer Res 2009; 15: 6980-6.
5. Cristofanilli M, Budd GT, Ellis MJ*, et al.* Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004; 351: 781-91.
6. Gupta V, Jafferji I, Garza M*, et al.* ApoStream(), a new dielectrophoretic device for antibody independent isolation and recovery of viable cancer cells from blood. Biomicrofluidics 2012; 6: 24133.
7. Alix-Panabieres C, Pantel K. Circulating tumor cells: liquid biopsy of cancer. Clin Chem 2013; 59: 110-8.
8. Lin H, Balic M, Zheng S, Datar R, Cote RJ. Disseminated and circulating tumor cells: Role in effective cancer management. Crit Rev Oncol Hematol 2011; 77: 1-11.
9. Went PT, Lugli A, Meier S*, et al.* Frequent EpCam protein expression in human carcinomas. Hum Pathol 2004; 35: 122-8.
10. Mani SA, Guo W, Liao MJ*, et al.* The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008; 133: 704-15.
11. Hoegg S, Meyer A. Hox clusters as models for vertebrate genome evolution. Trends Genet 2005; 21: 421-4.
12. Scott MP. A rational nomenclature for vertebrate homeobox (HOX) genes. Nucleic Acids Res 1993; 21: 1687-8.
13. Phelan ML, Sadoul R, Featherstone MS. Functional differences between HOX proteins conferred by two residues in the homeodomain N-terminal arm. Mol Cell Biol 1994; 14: 5066-75.
14. Pinsonneault J, Florence B, Vaessin H, McGinnis W. A model for extradenticle function as a switch that changes HOX proteins from repressors to activators. EMBO J 1997; 16: 2032-42.
15. Alharbi RA, Pettengell R, Pandha HS, Morgan R. The role of HOX genes in normal hematopoiesis and acute leukemia. Leukemia 2013; 27: 1000-8.
16. Shah N, Sukumar S. The Hox genes and their roles in oncogenesis. Nat Rev Cancer 2010; 10: 361-71.
17. Kroon E, Krosl J, Thorsteinsdottir U*, et al.* Hoxa9 transforms primary bone marrow cells through specific collaboration with Meis1a but not Pbx1b. EMBO J 1998; 17: 3714-25.
18. Morgan R, Boxall A, Harrington KJ*, et al.* Targeting the HOX/PBX dimer in breast cancer. Breast Cancer Res Treat 2012; 136: 389-98.
19. Morgan R, Boxall A, Harrington KJ*, et al.* Targeting HOX transcription factors in prostate cancer. BMC Urol 2014; 14: 17.
20. Morgan R, Pirard PM, Shears L*, et al.* Antagonism of HOX/PBX dimer formation blocks the in vivo proliferation of melanoma. Cancer Res 2007; 67: 5806-13.
21. Morgan R, Plowright L, Harrington KJ, Michael A, Pandha HS. Targeting HOX and PBX transcription factors in ovarian cancer. BMC Cancer 2010; 10: 89.
22. Plowright L, Harrington KJ, Pandha HS, Morgan R. HOX transcription factors are potential therapeutic targets in non-small-cell lung cancer (targeting HOX genes in lung cancer). Br J Cancer 2009; 100: 470-5.

23. Shears L, Plowright L, Harrington K, Pandha HS, Morgan R. Disrupting the interaction between HOX and PBX causes necrotic and apoptotic cell death in the renal cancer lines CaKi-2 and 769-P. J Urol 2008; 180: 2196-201.
24. Darda L, Hakami F, Morgan R*, et al.* The Role of HOXB9 and miR-196a in Head and Neck Squamous Cell Carcinoma. PLoS One 2015; 10: e0122285.
25. Rubin E, Wu X, Zhu T*, et al.* A role for the HOXB7 homeodomain protein in DNA repair. Cancer Res 2007; 67: 1527-35.
26. Kachgal S, Mace KA, Boudreau NJ. The dual roles of homeobox genes in vascularization and wound healing. Cell Adh Migr 2012; 6: 457-70.
27. Liao WT, Jiang D, Yuan J*, et al.* HOXB7 as a prognostic factor and mediator of colorectal cancer progression. Clin Cancer Res 2011; 17: 3569-78.
28. Bitu CC, Destro MF, Carrera M*, et al.* HOXA1 is overexpressed in oral squamous cell carcinomas and its expression is correlated with poor prognosis. BMC Cancer 2012; 12: 146.
29. De Souza Setubal Destro MF, Bitu CC, Zecchin KG*, et al.* Overexpression of HOXB7 homeobox gene in oral cancer induces cellular proliferation and is associated with poor prognosis. Int J Oncol 2010; 36: 141-9.
30. Hakami F, Darda L, Stafford P*, et al.* The roles of HOXD10 in the development and progression of head and neck squamous cell carcinoma (HNSCC). Br J Cancer 2014; 111: 807-16.
31. Tucci R, Campos MS, Matizonkas-Antonio LF*, et al.* HOXB5 expression in oral squamous cell carcinoma. J Appl Oral Sci 2011; 19: 125-9.
32. Yamatoji M, Kasamatsu A, Yamano Y*, et al.* State of homeobox A10 expression as a putative prognostic marker for oral squamous cell carcinoma. Oncol Rep 2010; 23: 61-7.
33. Sharpe DJ, Orr KS, Moran M*, et al.* POU2F1 activity regulates HOXD10 and HOXD11 promoting a proliferative and invasive phenotype in head and neck cancer. Oncotarget 2014; 5: 8803-15.
34. Moon SM, Ahn MY, Kwon SM*, et al.* Homeobox C5 expression is associated with the progression of 4-nitroquinoline 1-oxide-induced rat tongue carcinogenesis. J Oral Pathol Med 2012; 41: 470-6.
35. Moon SM, Kim SA, Yoon JH, Ahn SG. HOXC6 is deregulated in human head and neck squamous cell carcinoma and modulates Bcl-2 expression. J Biol Chem 2012; 287: 35678-88.
36. Platais C, Hakami F, Darda L*, et al.* The role of HOX genes in head and neck squamous cell carcinoma. J Oral Pathol Med 2015.
37. Chen KN, Gu ZD, Ke Y*, et al.* Expression of 11 HOX genes is deregulated in esophageal squamous cell carcinoma. Clin Cancer Res 2005; 11: 1044-9.
38. Liu DB, Gu ZD, Cao XZ, Liu H, Li JY. Immunocytochemical detection of HoxD9 and Pbx1 homeodomain protein expression in Chinese esophageal squamous cell carcinomas. World J Gastroenterol 2005; 11: 1562-6.
39. Han Y, Tu WW, Wen YG*, et al.* Identification and validation that up-expression of HOXA13 is a novel independent prognostic marker of a worse outcome in gastric cancer based on immunohistochemistry. Med Oncol 2013; 30: 564.
40. Zhang Q, Jin XS, Yang ZY*, et al.* Upregulated Hoxc6 expression is associated with poor survival in gastric cancer patients. Neoplasma 2013; 60: 439-45.
41. Kanai M, Hamada J, Takada M*, et al.* Aberrant expressions of HOX genes in colorectal and hepatocellular carcinomas. Oncol Rep 2010; 23: 843-51.
42. Gray S, Pandha HS, Michael A, Middleton G, Morgan R. HOX genes in pancreatic development and cancer. JOP 2011; 12: 216-9.
43. Abe M, Hamada JI, Takahashi O*, et al.* Disordered expression of HOX genes in human non-small cell lung cancer. Oncology Reports 2006; 15: 797-802.
44. Calvo R, West J, Franklin W*, et al.* Altered HOX and WNT7A expression in human lung cancer. Proc Natl Acad Sci U S A 2000; 97: 12776-81.

45. Xiao F, Bai Y, Chen Z*, et al.* Downregulation of HOXA1 gene affects small cell lung cancer cell survival and chemoresistance under the regulation of miR-100. Eur J Cancer 2014; 50: 1541-54.
46. Zhan J, Wang P, Niu M*, et al.* High expression of transcriptional factor HoxB9 predicts poor prognosis in patients with lung adenocarcinoma. Histopathology 2014.
47. Hur H, Lee JY, Yun HJ, Park BW, Kim MH. Analysis of HOX gene expression patterns in human breast cancer. Mol Biotechnol 2014; 56: 64-71.
48. Cantile M, Cindolo L, Napodano G, Altieri V, Cillo C. Hyperexpression of locus C genes in the HOX network is strongly associated in vivo with human bladder transitional cell carcinomas. Oncogene 2003; 22: 6462-8.
49. Marra L, Cantile M, Scognamiglio G*, et al.* Deregulation of HOX B13 expression in urinary bladder cancer progression. Curr Med Chem 2013; 20: 833-9.
50. Jung C, Kim RS, Zhang HJ, Lee SJ, Jeng MH. HOXB13 induces growth suppression of prostate cancer cells as a repressor of hormone-activated androgen receptor signaling. Cancer Res 2004; 64: 9185-92.
51. Miller GJ, Miller HL, van Bokhoven A*, et al.* Aberrant HOXC expression accompanies the malignant phenotype in human prostate. Cancer Res 2003; 63: 5879-88.
52. Jiang Y, Chu Y, Tang W*, et al.* Transcription factor WT1 and promoter CpG hypomethylation coactivate HOXA10 expression in ovarian cancer. Curr Pharm Des 2014; 20: 1647-54.
53. Kelly ZL, Michael A, Butler-Manuel S, Pandha HS, Morgan RG. HOX genes in ovarian cancer. J Ovarian Res 2011; 4: 16.
54. Stavnes HT, Holth A, Don T*, et al.* HOXB8 expression in ovarian serous carcinoma effusions is associated with shorter survival. Gynecol Oncol 2013; 129: 358-63.
55. Morgan R, Whiting K. Differential expression of HOX genes upon activation of leukocyte sub-populations. Int J Hematol 2008; 87: 246-9.
56. Lawrence HJ, Sauvageau G, Ahmadi N*, et al.* Stage- and lineage-specific expression of the HOXA10 homeobox gene in normal and leukemic hematopoietic cells. Exp Hematol 1995; 23: 1160-6.

Figure 1

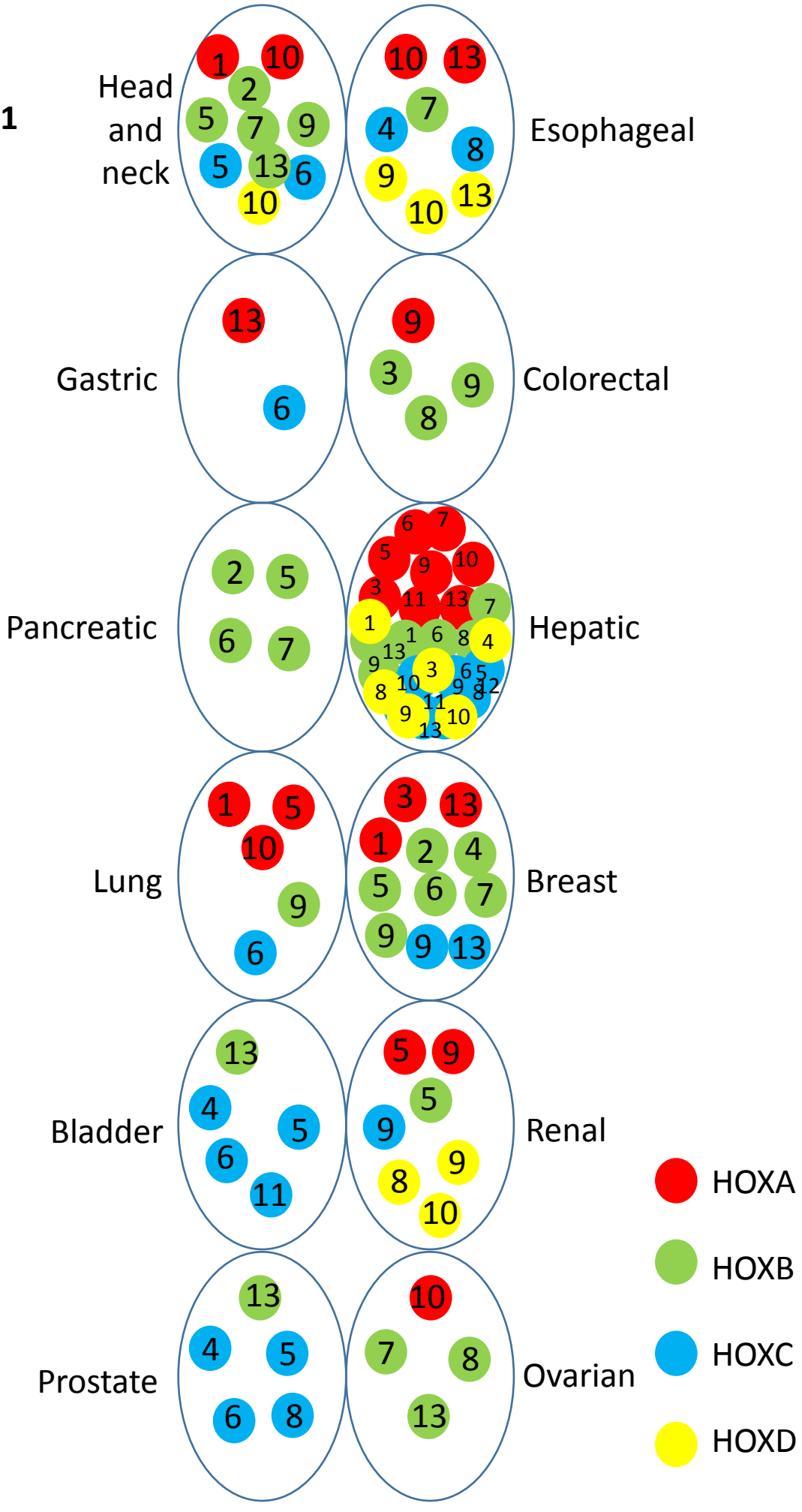
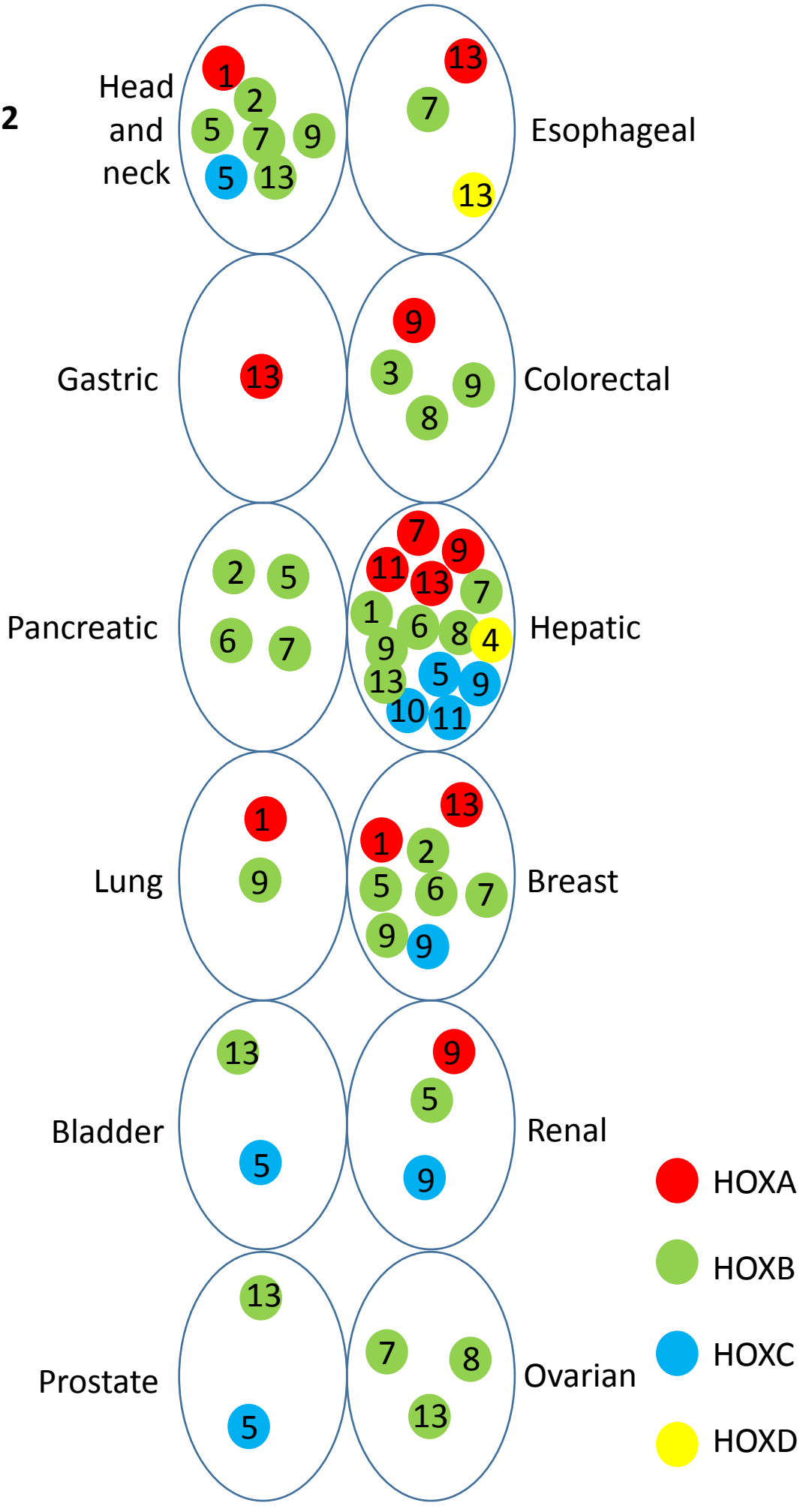

Head and neck
Esophageal
Gastric
Colorectal
Pancreatic
Hepatic
Lung
Breast
Bladder
Renal
Prostate
Ovarian
HOXA
HOXB
HOXC
HOXD

Figure 2


Head and neck
1
2
5
7
9
5
13
Esophageal
13
7
13
Gastric
13
Colorectal
9
3
9
8
Pancreatic
2
5
6
7
Hepatic
7
9
11
13
7
1
6
8
4
9
13
5
9
10
11
Lung
1
9
Breast
13
1
2
5
6
7
9
9
Bladder
13
5
Renal
9
5
9
Prostate
13
5
Ovarian
7
8
13
HOXA
HOXB
HOXC
HOXD